

Spermatozoal morphology of the freshwater anomuran *Aegla longirostri* Bond-Buckup & Buckup, 1994 (Crustacea: Decapoda: Aeglidae) from South America

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Abstract.—Unique sperm morphology is described for *Aegla longirostri* Bond-Buckup & Buckup, 1994, a representative of the freshwater anomuran family Aeglidae from South America. Comparisons of the spermatozoal ultrastructure of this species with that described for other anomurans indicate that *A. longirostri* has a distinct suite of spermatozoal characters. Within the Anomura, the aeglids share more spermatozoal characters with the superfamily Lomoidea, represented by the monotypic Australian endemic genus, *Lomis*, than to any previously described representative from the Galattheoidea, Hippoidea, or Paguroidea. A more basal ancestry, with an independent evolutionary lineage, within the Anomura is postulated for the Aeglidae. A superficial resemblance of the spermatozoal ultrastructure of *A. longirostri* to that described for a palinurid lobster, *Jasus*, and a thalassinidean mud shrimp, *Neaxius*, is also noted.

The endemic South American freshwater anomuran crab family Aeglidae Dana, 1852, currently consists of the genus *Aegla* Leach, 1820, with more than 60 species, and the two fossil genera *Haumuriaegla* Feldmann, 1984 and *Protaegla* Feldmann et al., 1998. The taxonomy and systematics of the genus *Aegla* has been adequately summarized by Martin & Abele (1988). The family Aeglidae is one of 14 anomuran families, and is currently placed in the superfamily Galattheoidea, with the families Chirostylidae, Galatheidae, and Porcellanidae (Bowman & Abele 1982, Martin & Davis 2001).

Following the classification suggested by McLaughlin (1983b) and Martin & Davis (2001), but acknowledging the existence of alternative classifications with an additional superfamily Coenobitoidea (Bowman & Abele 1982, Forest 1987), we concur that

the Anomura consists of only four superfamilies, the Lomoidea, Hippoidea, Paguroidea, and the Galattheoidea. The relationships of the Aeglidae to other anomuran families continues to be equivocal. Milne-Edwards & Bouvier (1894) concluded that the aeglids were derived from marine hermit crabs, and placed them on a direct lineage to the galatheids (Galatheidae). Later workers (Martin 1985, 1989; Martin & Abele 1988) linked the aeglids with the hermit crabs (Paguroidea) rather than with the galatheids, but a sister-group relationship with the Galattheoidea has also been suggested (Martin & Abele 1986). The similarity of aeglids, in gross external morphology, to the marine galatheids and chirostylids is the source of much of this confusion and resulting speculation. Representatives of the Aeglidae were absent from a recent phylogenetic analysis of anomuran relation-

ships based on reproductive characters (Tudge 1997b), but were included in the carcinization matrix and tree of McLaughlin & Lemaitre (1997).

Twelve anomuran families have previously been investigated for sperm and spermatophore morphology (Tudge et al. 1999, 2001; Jamieson & Tudge 2000, and references therein). The spermatozoal ultrastructure of representatives of the remaining two families, the Aeglididae and Pylojacquesidae (the latter a monotypic hermit crab family recently established by McLaughlin & Lemaitre 2001), have yet to be described and illustrated. This paper describes for the first time the ultrastructure of the spermatozoa of a species of Aeglididae, *Aegla longirostri* Bond-Buckup & Buckup, 1994, and provides an additional suite of characters that might be useful in phylogenetic studies of the family. A comparison of the sperm morphology of *A. longirostri* to that already described for anomurans and other decapods is also made.

Methods

A single male specimen of *Aegla longirostri* was collected from the Carreiro River, in the State of Rio Grande do Sul, Brazil, on 30 October 2000. The gonads were removed and fixed in 3% glutaraldehyde in phosphate buffer and posted to the National Museum of Natural History, Smithsonian Institution, Washington, D.C. A squash in phosphate buffer was made upon receipt, and the tissue was examined and photographed through an Olympus BH2 Nomarski interference contrast microscope and attached Olympus OM-2 camera. The remainder of the tissue was processed for TEM as outlined below.

After the initial glutaraldehyde fixation and phosphate buffer wash, the remainder of the fixation protocol was carried out in a Lynx-el. Microscopy Tissue Processor. Portions of the vas deferens were washed in phosphate buffer (three washes of 15 min), postfixed in phosphate buffered 1%

osmium tetroxide for 80 min, similarly washed in buffer, and dehydrated through ascending concentrations of ethanol (20–100%). After infiltrating and embedding in Spurr's epoxy resin (Spurr 1969), thin sections (50–80 nm thick) were cut on a LKB 2128 μm IV microtome with a diamond knife. Sections were placed on carbon-stabilized collodion-coated 200 μm mesh copper grids and stained in 6% aqueous uranyl acetate for 30 sec; rinsed in distilled water; stained with Reynold's lead citrate (Reynolds 1963) for four minutes; and further stained in uranyl acetate for two minutes before a final rinse in distilled water (Daddow 1986). Micrographs were taken on a Hitachi 300 transmission electron microscope at 80 kV.

Spermatozoal Ultrastructure

When viewed at the TEM level the spermatozoa of *Aegla longirostri* are spherical to ovoid cells (polymorphic in oblique section) with the cell body divided into two hemispheres (Figs. 1, 2A, B). The upper, or apical, hemisphere is composed of the cytoplasm and the spherical acrosome vesicle, while the lower, or basal, hemisphere is composed of nuclear material. The sperm cells show some variation in their dimensions, but are 4.6 μm wide ($n = 15$; range = 4.1–5 μm) and 4.1 μm in height ($n = 11$; range = 3.4–4.5 μm). This latter measurement was taken through the acrosomal axis. The small acrosome vesicle is 1.5 μm wide ($n = 12$; range = 1.1–1.8 μm) and 1.4 μm in height ($n = 5$; range = 1.1–1.9 μm) (Figs. 1–3).

The acrosome vesicle forms a conspicuous, electron-dense, ring-like structure at the pole of the apical hemisphere. In longitudinal section (LS), the acrosome vesicle appears as two opposing "half-moon" shapes, with an electron-pale column intervening (Fig. 1B). In oblique sections (some close to transverse), the acrosome vesicle is an irregular, electron-dense ring, with a crenulated, and sometimes vesicular, ap-

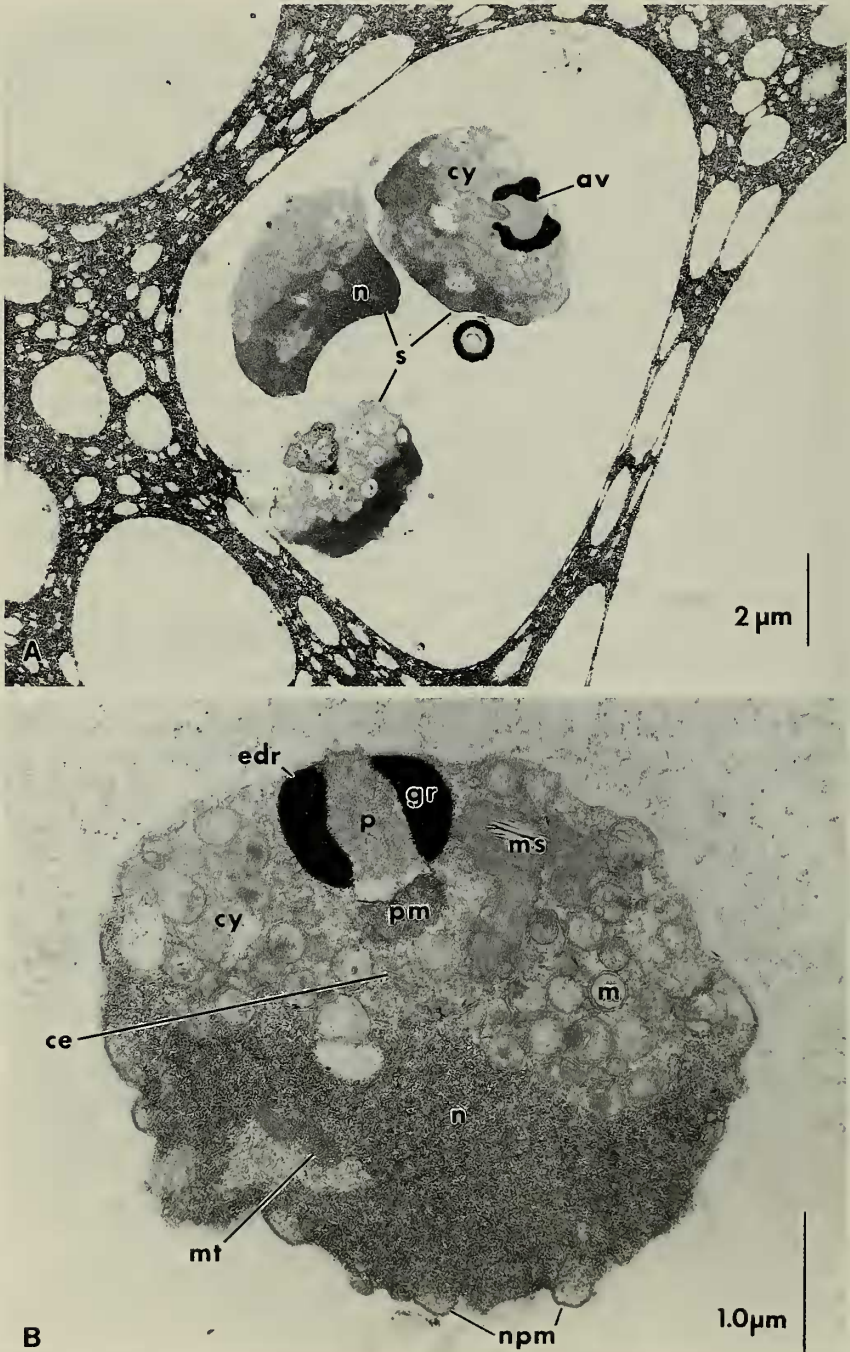


Fig. 1. Transmission electron micrographs of spermatozoa of *Aegla longirostri* Bond-Buckup & Buckup, 1994. A, Three sperm cells in various sections; B, LS of spermatozoon. Abbreviations: av, acrosome vesicle; ce, centriole; cy, cytoplasm; edr, electron-dense acrosome region; gr, granular acrosome region; m, mitochondrion; ms, membrane system; mt, microtubular bundles; n, nucleus; npm, nucleo-plasma membrane; p, perforatorial column; pm, periacrosomal material; s, spermatozoa. Scale bars as indicated.

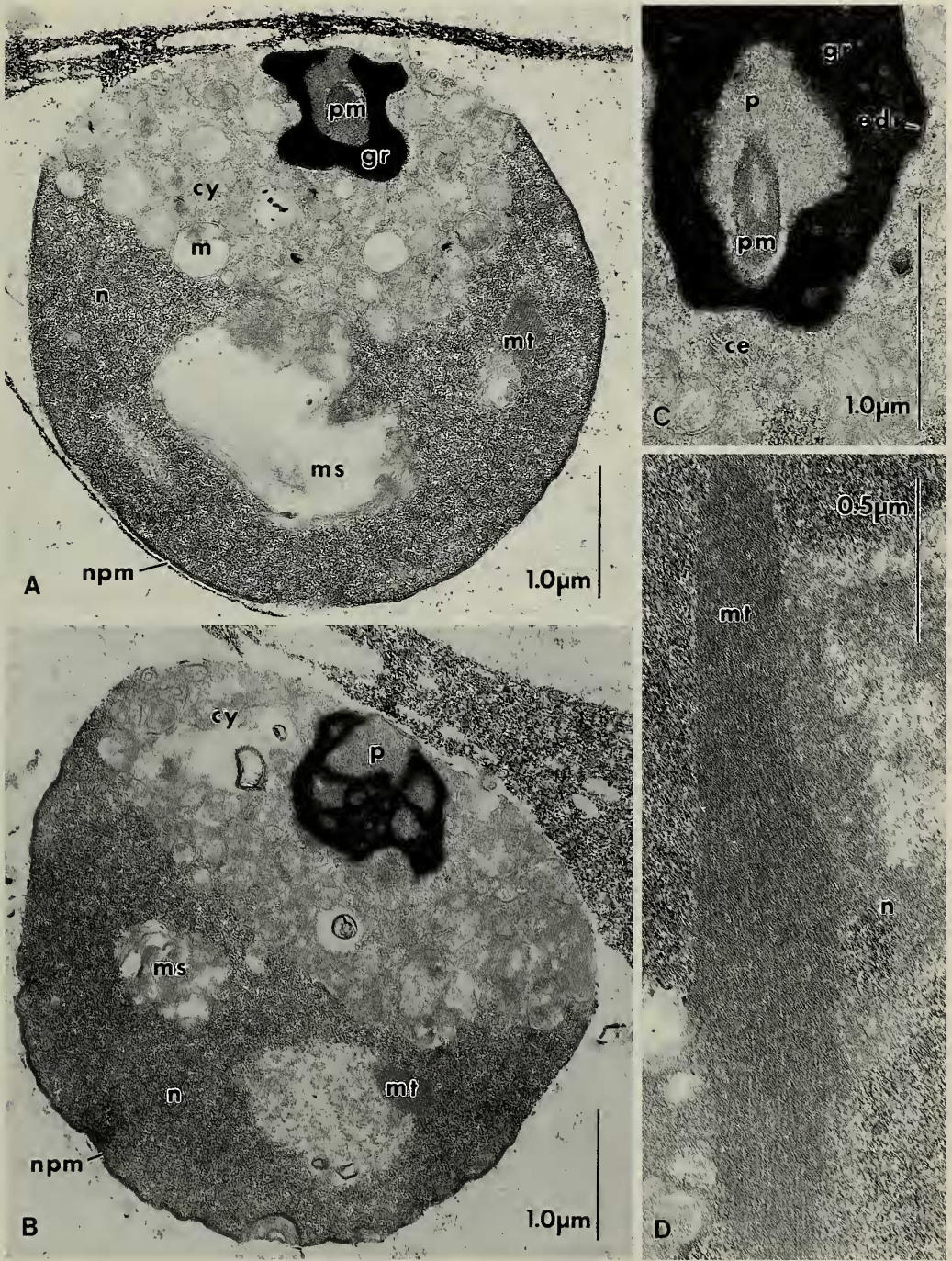


Fig. 2. Transmission electron micrographs of spermatozoa of *Aegla longirostri* Bond-Buckup & Buckup, 1994. A, B, Oblique sections of spermatozoa showing variable amounts of cytoplasm and nucleus; C, Detail of oblique section through the acrosome vesicle; D, Detail of LS of microtubular bundle. Abbreviations: see fig. 1. Scale bars as indicated.

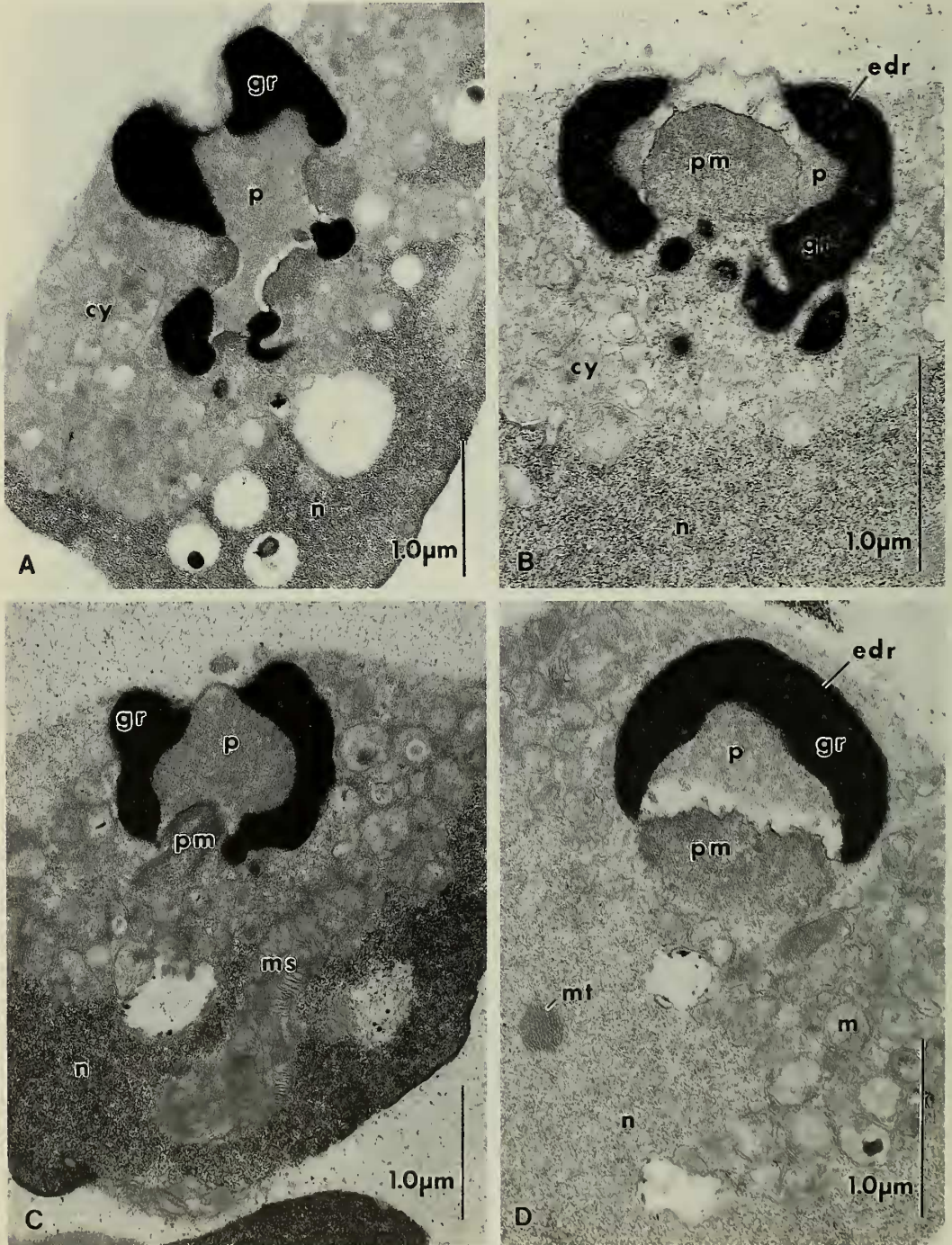


Fig. 3. Transmission electron micrographs of spermatozoa of *Aegla longirostri* Bond-Buckup & Buckup, 1994. A–D, Oblique sections of acrosome vesicle detail. Abbreviations: see fig. 1. Scale bars as indicated.

pearance (Figs. 2A, B, 3). Closer inspection of the electron-dense part of the acrosome reveals a thin, extremely electron-dense region just inside the acrosome vesicle membrane. This thin, dark, strip envelops the entire acrosome vesicle, with the exception of the posterior and anterior electron-lucent zones. Interior to this electron-dense strip, the acrosome vesicle contents are less electron-dense and are coarsely granular, with small electron-opaque patches (Figs. 1, 2A-C, 3). In one section, these lighter patches were seen to be larger lacunae within the darker matrix (Fig. 2B). The center of the acrosome vesicle is occupied by a coarsely granular, electron-pale cylindrical column, here termed the perforatorial column. This paler region separates the dense part of the acrosome vesicle into the two crescent shapes in LS (Fig. 1B) and forms the center of a dense ring in oblique and near-transverse sections (Figs. 2A, C, 3A-C). The upper and most basal regions of the perforatorial column are bound by the acrosome vesicle membrane only, and posteriorly may be separated from the membrane by a translucent area (Figs. 1B, 3A, D).

Immediately subjacent to the acrosome vesicle is a subacrosomal (periacrosomal) region, clearly demarcated from the surrounding cytoplasm by its more coarsely granular appearance and greater electron density (Figs. 1, 2A, C, 3B-D). This periacrosomal region is not membrane-bound, but closely abuts the posterior membrane of the acrosome vesicle. In some sections this denser periacrosomal material appears to partially penetrate the perforatorial column as thick protrusions (Figs. 1A, 2A, C, 3C).

The cytoplasm of the sperm cell occupies the majority of the apical hemisphere, with the exception of the acrosome vesicle (Figs. 1, 2A, B, 3). Organelles include many spherical, electron-pale, sparsely cristate mitochondria, some densely arranged membrane systems (Figs. 1B, 2A, B, 3C), and the occasional centriole (Fig. 2C); all of these are embedded in an irregular coarse granular matrix. No distinct membrane sep-

arates the cytoplasm from the more basal nuclear material.

The nucleus constitutes approximately 50% of the cell volume and occupies the basal hemisphere of the spermatozoa (Figs. 1B, 2A, B). The nuclear material is moderately electron-dense (more so than the adjacent cytoplasm), homogeneously granular, and surrounded externally by a thickened double membrane (Figs. 1B, 2A, B, 3A, C). This obvious membrane is composed of both the nuclear membrane and the plasma membrane (termed the nucleo-plasma membrane). The homogeneous nucleus may have large electron-lucent spaces evident in some sections. These spaces may contain small, extremely electron-dense spots (Fig. 3A), regularly arranged thin membranes (Figs. 2A, B, 3C), sparse granular material (Figs. 1B, 2A, B), or no distinguishable material (Figs. 3A, C, D). It is not known if these electron-lucent gaps are artifacts of fixation or characteristic features of the spermatozoa. They appear, in some form or another, in the majority of sperm cells observed, and some were even infrequently seen in the cytoplasm (Figs. 1B, 2B, 3A). Besides these inclusions, the only other structures present in the nuclear material are tight bundles of microtubules. These microtubule bundles were seen in longitudinal, oblique, and transverse section in various sperm cells and are deduced to be internalized microtubular arms (Figs. 1B, 2A, B, D, 3D). From one to three long arms were observed in *Aegla longirostri*, and also in *A. rostrata* Jara, 1977 (unpublished observation), at the light microscope level, but they were not apparent at the TEM level. The bundles of microtubules appeared to be restricted to the nucleus, with none observed in the cytoplasmic region.

Discussion

When comparisons are made to the previously described spermatozoa in the Anomura (Tudge et al. 1999, 2001; Jamieson &

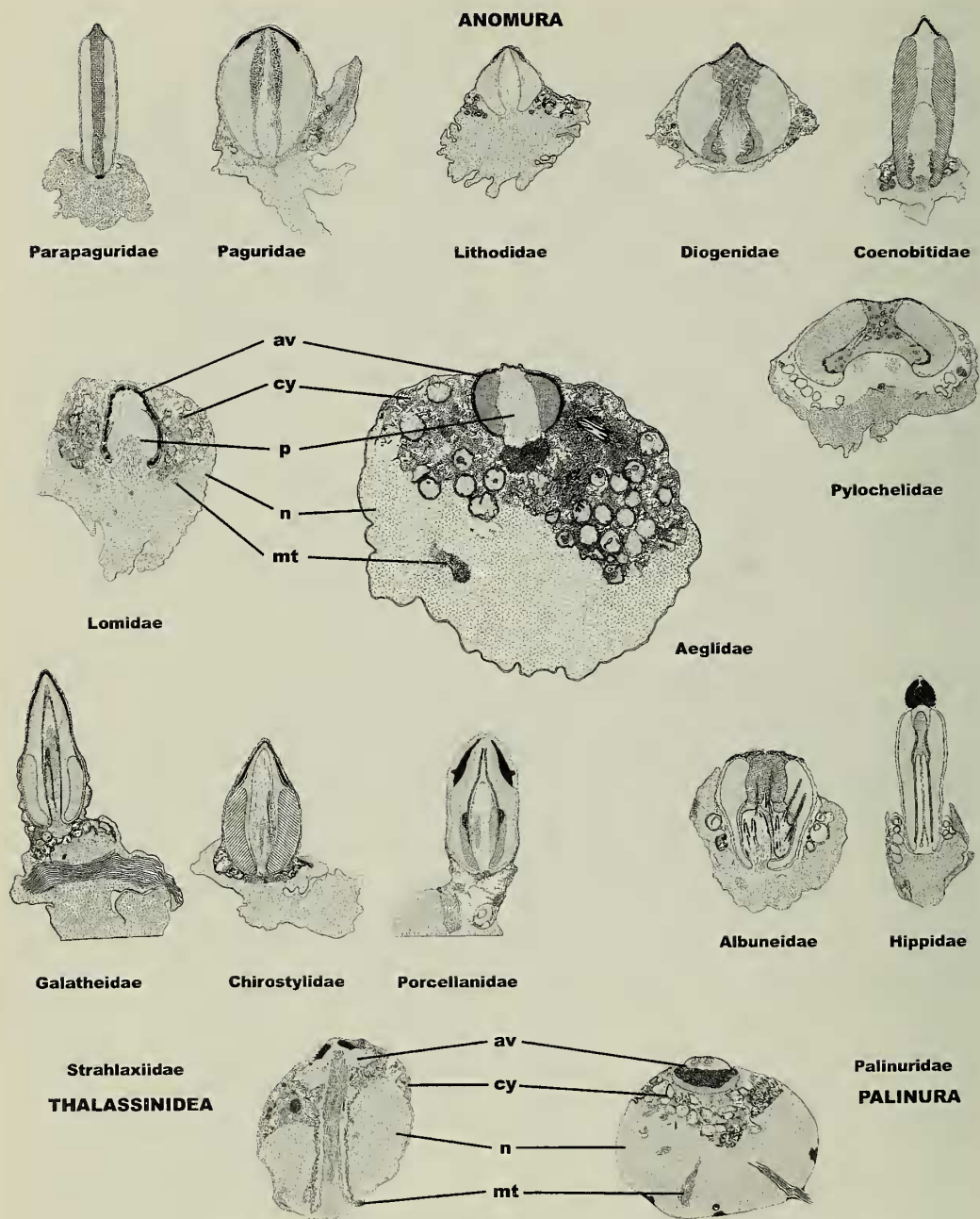


Fig. 4. Semidiagrammatic longitudinal sections of spermatozoa from 13 anomuran, thalassinidean, and palinurid families for comparison of gross morphologies. Parapaguridae: *Sympagurus* sp.; Paguridae: *Pagurus bernhardus* (Linnaeus, 1758); Lithodidae: *Lithodes maja* (Linnaeus, 1758); Diogenidae: *Clibanarius longitarsus* (De Haan, 1849); Coenobitidae: *Coenobita purpurea* Stimpson, 1858; Pylochelidae: *Pylocheles (Bathyecheles)* sp.; Lomidae: *Lomis hirta* (Lamarck, 1818); Aeglidae: *Aegla longirostri* Bond-Buckup & Buckup, 1994; Galatheidae: *Munidopsis* sp.; Chirostylidae: *Eumunida sternomaculata* St. Laurent & Macpherson, 1990; Porcellanidae: *Petrolisthes lamarkii* (Leach, 1820); Albuneidae: *Albunea marquisiana* Boyko, 2000; Hippidae: *Emerita talpoida* (Say, 1817); Strahlaxiidae: *Neaxius glyptocercus* (Von Martens, 1868); Palinuridae: *Jasus novaehollandiae* Holthuis, 1963. Abbreviations: see Fig. 1. Not to scale. (Parapaguridae, Diogenidae, Lomidae, Chirostylidae, Strahl-

Tudge 2000, and references therein), it is evident that the sperm cells observed in *Aegla longirostri* are unique (see Fig. 4). The spermatozoal morphology of *A. longirostri* differs significantly from any of the 12 other investigated families in the Anomura. Instead, the overall morphology of the spermatozoa of *A. longirostri* most closely resembles that described for the Australian endemic hairy stone crab, *Lomis hirta* (Lamarck, 1818), of the superfamily Lomoidea, by Tudge (1997a). Similarities between *Aegla* and *Lomis* spermatozoa include the irregular number of microtubular arms (from none to three), the division of the sperm cell contents into roughly equal hemispheres with the cytoplasm and acrosome in one and the nucleus in the other, many small mitochondria, and a small spherical to ovoid acrosome vesicle embedded in the cytoplasm (Figs. 1B, 4). Some shared ultrastructural features of the acrosome include an electron-pale perforatorial column penetrating the entire acrosome, and the outer electron-dense region (=operculum?) encircling the acrosome vesicle and having a crenulated, and somewhat lacunate, appearance in oblique and transverse sections. Two notable differences are that the outer dense region of the acrosome covers the apex in *Lomis*, whereas it is open in *Aegla*, and that the microtubular bundles are present in both the cytoplasm and nucleus in *Lomis* (see Tudge 1997a), while being restricted to the nucleus in *Aegla*.

Outside of the Anomura there are some general similarities of the spermatozoa (Figs. 1B, 4) of *Aegla longirostri* to those of some Palinura (Palinuridae) and Thalassinidea (Strahlaxiidae) (Tudge 1995a, 1995b; Tudge et al. 1998). Of particular note is the fact that the periacrosomal material in the spermatozoa of the palinurid

Jasus novaehollandiae Holthuis, 1963, penetrates the acrosome vesicle as blunt projections (Tudge et al. 1998) reminiscent of the condition observed in *A. longirostri*. The resemblance to some thalassinidean spermatozoa (Tudge 1995a, 1995b, 1997b; Jamieson & Tudge 2000) is more superficial and relates to the overall shape of the sperm cell, the common small and embedded acrosome, the large cytoplasmic region, and appearance of microtubules in the nucleus (a character also shared with *Jasus novaehollandiae*).

Spermatozoal comparisons across the Anomura clearly show the great dissimilarity of the sperm cell of *Aegla* to that of galatheoids, hippoids, or paguroids recorded to date (Fig. 4). No close spermatozoal affinities can be recognized with any of the known sperm of members of these three superfamilies. Based on sperm morphology alone of *Aegla* and *Lomis*, it would appear that the Lomidae may be closely related to the aeglids. The possibility of such a relationship has recently gained some support from comparisons of nuclear and mitochondrial genes within the Anomura (Morrison et al. 2002).

The current superfamily hierarchy in the Anomura is well supported by differences in reproductive characters derived from spermatophores and spermatozoa (Tudge 1992, 1995a, 1995b, 1997a, 1997b; Tudge et al. 1999, 2001; Jamieson & Tudge 2000). The spermatozoal morphology presented here provides some evidence that may help in the evaluation of whether the family Aeglidae can be elevated to superfamily status with close affinities to the Lomoidea. The unique nature of the Aeglidae to other galatheoid families has been previously documented based on external morphology and ecological data (Martin & Abele 1986,

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laxiidae after Tudge 1995b; Paguridae, Coenobitidae, Galatheidae, Porcellanidae after Tudge 1995a; Lithodidae, Palinuridae after Jamieson & Tudge 2000; Pylochelidae after Tudge et al. 2001; Albuneidae, Hippidae after Tudge et al. 1999).

1988). Recently, comparisons of the ribosomal 18s DNA gene among the Anomura has also indicated a clear separation of the aeglids from other galatheoid families (Pérez-Losada et al. 2002). As suggested for the Lomoidea by McLaughlin (1983a) and Tudge (1997a), an independent and more basal lineage for the Aeglidae within the Anomura is indicated by several disparate types of data, including spermatology. A more remote ancestor of aeglids than modern galatheoids seems possible considering the difficulty and inconsistency in identifying the closest sister taxon to these enigmatic freshwater crabs. Historically, aeglids have been linked with other Galathaeoidea (Martin & Abele 1986), the Paguroidea (Martin & Abele 1988), and now the Lomoidea.

This study of spermatozoa of *Aegla longirostri*, as well as previous investigations of the Aeglidae, show that they are distinct from other anomurans in morphology, ecology, spermatology, and molecular biology. Detailed and comprehensive phylogenetic investigations are needed of the Anomura to determine relationships among the various lineages, and elucidate aeglid affinities within the extant Decapoda.

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